Minimal persistent inflammation is also present in patients with seasonal allergic rhinitis

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Background: The allergic reaction is characterized by an inflammatory response, which is correlated to the allergen exposure, and is detectable in mite allergic patients, even when symptoms are absent.
Objective: The study was aimed at assessing the presence of inflammation in patients with pollen allergy during a long observation period.
Methods: Six patients, sensitized only to *Betula alba*, were enrolled. Evaluated parameters were (1) nasal symptoms, (2) inflammatory markers (ie, neutrophil and eosinophil number and intercellular adhesion molecule-1 expression on nasal epithelial cells), and (3) pollen count. Patients were examined during the pollen season every 4 days for 40 days and were observed 3 times after the pollen season.
Results: A significant inflammatory reaction was evident throughout the pollen season, even during the days with a low pollen count and low or absent symptoms.
Conclusions: The results of this study indicate that the global therapeutic strategy for allergic rhinitis should be revised and targeted to inflammatory phenomena rather than to symptoms alone. (J Allergy Clin Immunol 1999;104:54-7.)

Key words: *Pollen allergy, inflammation, adhesion molecules, eosinophils*

Abbreviations used
APAAP: Alkaline phosphatase–antialkaline phosphatase
ICAM-1: Intercellular adhesion molecule-1

It is generally agreed that allergic rhinitis is sustained by an inflammatory process. Mucosal eosinophilic infiltrate may actually be regarded as one of the hallmarks of allergic inflammation. Pathophysiologic mechanisms involved in eosinophil recruitment include release of mediators and cytokines, which chemoattract, activate, and prolong survival, and adhesion molecule machinery activation.[1]

Eosinophils express some adhesion molecules on their surface, including the β_2-integrins leukocyte function–associated molecule-1 and membrane attack complex-1, which are the counterreceptors of intercellular adhesion molecule-1 (ICAM-1), expressed on the epithelial cells of allergic subjects.[2] Epithelial cells of allergic subjects express ICAM-1 early on allergen challenge; thus this phenomenon may, at least partially, account for mucosal leukocyte infiltration.[3] The relevance of these data has been confirmed by detecting ICAM-1 expression during natural exposure to the allergens.[4] Moreover, if allergen exposure is prolonged (as in mite allergy), allergic inflammation is always detectable, even when symptoms are not present. A "minimal persistent inflammation" has been demonstrated at both conjunctival and nasal levels in symptom-free rhinitic patients with mite allergy.[5] Therefore constant exposure to the allergen, although it does not always induce symptoms, is still able to promote and maintain inflammation, detectable for instance by monitoring ICAM-1 expression on epithelial cells or inflammatory cell infiltrate in the mucosa.

We therefore designed a study aimed at assessing the presence of inflammation in patients with pollen allergy during a long observation period.

METHODS

The study was designed to evaluate patients with seasonal allergic rhinitis just before, during, and after the pollen season.

Six patients were enrolled (2 men and 4 women, mean age 29 years, range 18-33 years). All of them were sensitised only to *Betula alba*, as confirmed by history, skin prick test, specific serum IgE, and specific nasal challenge (confirming that all subjects had a low grade of sensitization); also they had required only a few drug prescriptions, on demand, during the previous pollen seasons.

The protocol was approved by the Ethics Committee and informed consent was required before enrolment.

Patients were excluded if they had a concurrent upper airway infection assessed by clinical (presence of sore throat, nasal mucopurulent discharge, headache with or without fever, pharyngeal redness, etc) and cytologic (neutrophilia, cytociliophoria, etc) parameters, according to a previous study.[6] Moreover, patients with anatomic nasal problems or with other significant medical problems were not enrolled, nor were patients receiving specific immunother-

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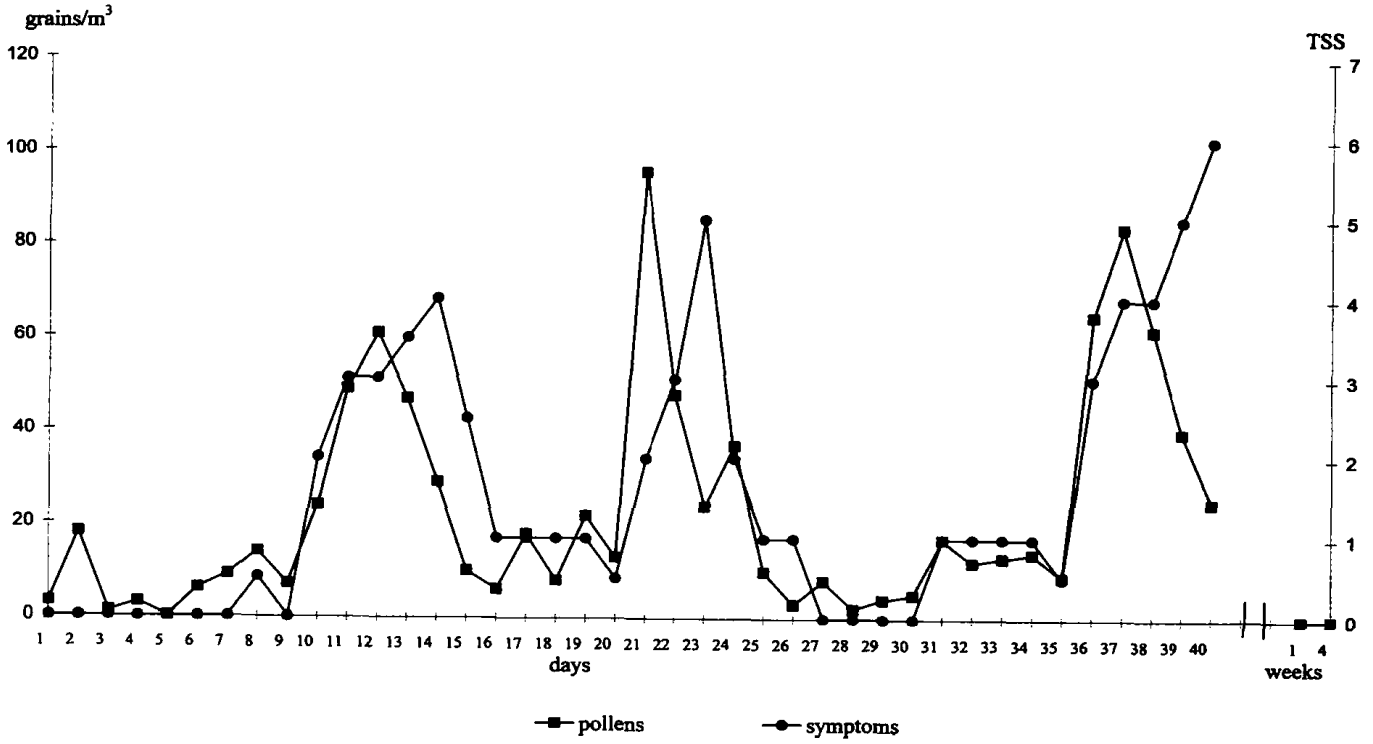

FIG 1. Mode of daily total symptom scores and pollen counts.

apy. No drug was permitted 1 month before and during the study. The rescue medication was the vasoconstrictor oxymetazoline, which does not exert antiallergic activity.[4]

Evaluated parameters were (1) nasal symptoms (nasal itching and obstruction, sneezing, and rhinorrhea scored with an arbitrary scale at 4 points: 0 = absent, 1 = mild, 2 = moderate, 3 = severe) recorded daily in the evening on a diary card, (2) inflammatory markers (ie, neutrophil and eosinophil number and ICAM-1 expression on nasal epithelial cells), and (3) pollen count.

A clinical examination was performed on all patients just before the pollen season; they were then examined during the pollen season every 4 days for 40 days and were observed 3 times after the pollen season. A nasal scraping was performed at each visit.

The pollen count was taken using a pollen-trap device.

Cytologic assessment

Nasal scraping was obtained according to previous reports.[3-5] Briefly, the cotton tip was immersed after the scraping in PBS in a plastic tray and transferred to a 10-mL polypropylene tube. The recovered fluids were then centrifuged at 1400*g* for 10 minutes and each pellet was resuspended in PBS solution (2 mL); cell suspensions were filtered to reduce the quantity of mucus and cytospin slides were prepared with use of standard techniques.

The differentiation among eosinophils, neutrophils, and epithelial cells was made by May-Grünwald/Giemsa staining. The number of inflammatory cells (neutrophils and eosinophils) was considered as a total number of any cell type per each microscope field: the data were expressed as a mean of 10 fields.

Immunocytochemistry

The immunoenzymatic alkaline phosphatase–monoclonal antialkaline phosphatase (APAAP) complex procedure was used.[3-5]

Specimens were air dried at room temperature for 30 minutes and submitted to 1:50 dilution of purified CD54 mAb (1 mg/mL, 84H10, IgG_1, Immunotech, Marseille, France) or to 1:100 dilution of anticytokeratin mAb (aCK19, IgG_1, DAKO, Milan, Italy) as a marker of epithelial cells. After they were washed in PBS, pH 7.6, samples were incubated with rabbit antimouse Ig, followed by APAAP complex. Afterward, specimens were incubated in substrate solution containing basic new fuchsin, napthol as biphosphate, and levamisole as an inhibitor of endogenous alkaline phosphatase (Sigma, St Louis). In control samples either mAb or antimouse Ig was omitted. As a negative isotype control for CD54 staining on epithelial cells, an anti–T-lymphocyte (CD3) mAb OKT3, IgG_1, (Ortho Diagnostic, Raritan, NJ) at a 1:20 dilution of the stock solution (provided by the manufacturer) was used. The dilutions were established on the basis of previous titration experiments. All preparations were counterstained with Carazzi's hematoxylin. The person measuring ICAM-1 was blinded to the timing of sample collection.

ICAM-1 expression on epithelial cells was expressed according to a 4-point rating scale, from 0 to 4, where 0 = no positive cells, 1 = mild positivity on 25% of epithelial cells, 2 = mild positivity on 75% of epithelial cells, 3 = intense positivity on 75% of epithelial cells, and 4 = very intense positivity on all epithelial cells, according to our previous reports.[3-5]

Statistical analysis

Statistical analysis was performed with use of the chi-square test and Fisher's test.

RESULTS

All enrolled patients completed the study, without concurrent respiratory infections.

Pollen counts

The daily pollen counts collected during the study are reported in Fig 1. There were some days with a very low pollen count because of weather conditions such as rain.

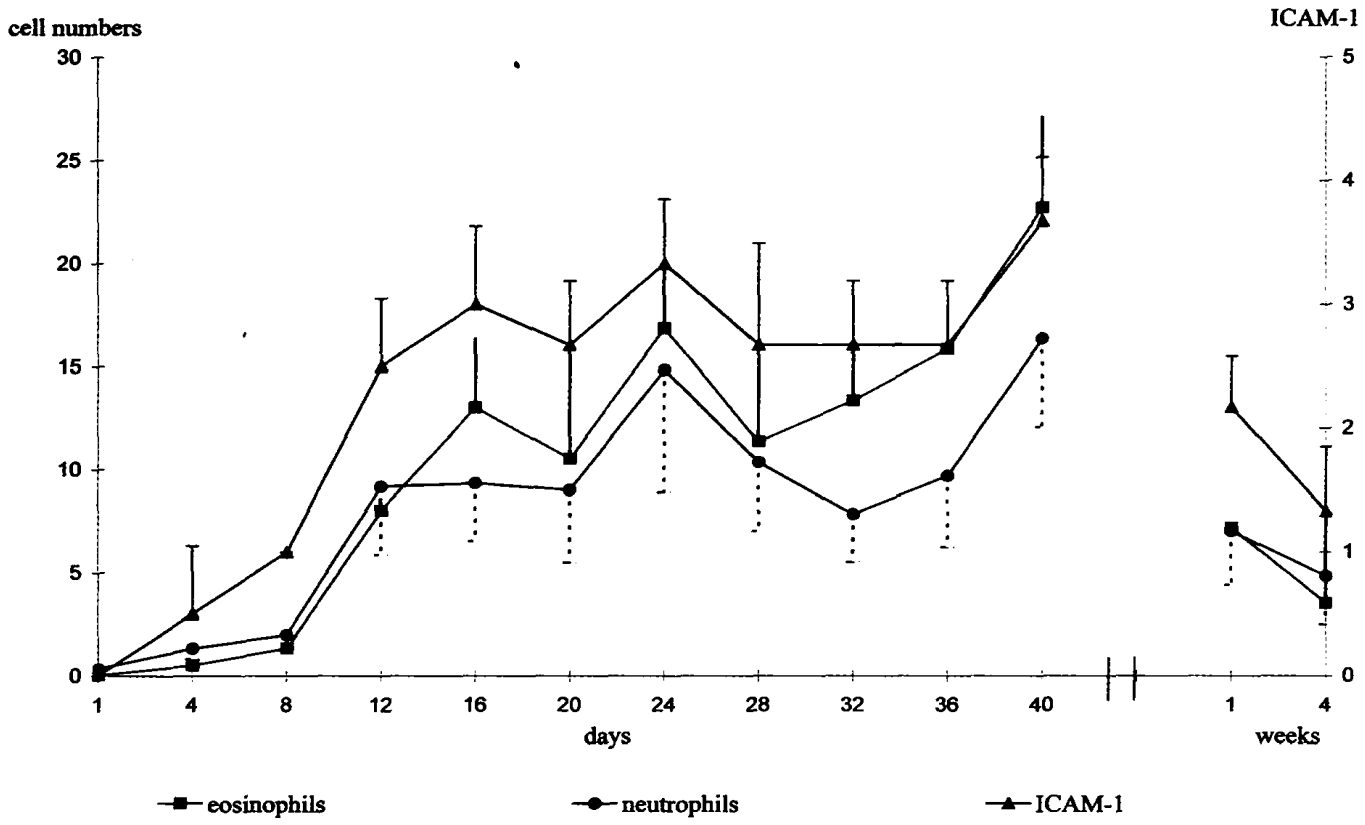

FIG 2. Eosinophil and neutrophil counts in nasal scrapings and ICAM-1 positivity on nasal epithelial cells during visits (±SD).

The *B alba* pollen season lasted for a short period (about one half month).

Symptoms

The total symptom score is reported in Fig 1. There are some days without symptoms, corresponding to low pollen counts.

Inflammatory parameters are reported Fig 2.

A significant inflammatory reaction (arbitrarily defined as more than 5 neutrophils, more than 1 eosinophil per field, and more than class 1 of ICAM-1 score[5]) is evident throughout the pollen season, even during the days with low pollen count and low or absent symptoms ($P = .034$, $F = 0.017$). After the pollen season inflammation persisted for 4 weeks.

DISCUSSION

It has been demonstrated that ICAM-1 expression on epithelial cells may be considered a marker of allergic inflammation[7]; in fact, its presence correlates with eosinophil infiltration resulting from exposure to the allergen. Moreover, ICAM-1 expression and eosinophil recruitment may be present without symptoms during allergen exposure, both with mite allergens[4] and pollen allergens, as reported in this study, although the number of subjects studied is small and the results may not be generalizable to all subjects with allergic rhinitis. However, the presence of a minimal persistent inflammation was detectable in the patients studied, exposed to the pollens, during symptomless days and immediately after the pollen season (until 4 weeks). This finding confirms previous studies showing a chronic state of allergic inflammation of the nasal mucosa, involving various inflammatory mediators, and measurable changes in the function of the nose and eustachian tube during seasonal pollen exposure.[8-11]

This may be considered another piece of the complex puzzle of allergic inflammation and it underlines that symptoms can no longer be considered the unique marker of allergic disease. In fact, both inflammation and hyperreactivity persist without symptoms until allergen exposure occurs[5] and immediately afterward.

Therefore symptoms may be envisaged as the "peak of the iceberg" of the allergic reaction, where inflammation and hyperreactivity represent the submerged reality. This evidence may represent an important clinical issue. Because avoiding pollen is not possible from a practical point of view, alternative pharmacologic strategies must be used. The results of this study combined with those of previous trials[6,12,13] indicate that the global therapeutic strategy for allergic rhinitis should be revised and aimed at inflammatory phenomena rather than at symptoms only. This could be achieved by monitoring allergic inflammation (eg, by nasal scraping, assessing eosinophil infiltration, as part of routine clinical practice) and by

using long-term treatment with anti-inflammatory agents protracted throughout the entire period of allergenic exposure rather than symptomatic treatment as recently recommended by some clinical experiences.[6,12,13] Regarding this, we previously demonstrated that for azelastine (a topical antihistamine with antiallergic activity) prolonged treatment during the entire pollen season is better than symptomatic use.[12] In addition, we demonstrated that continuous cetirizine treatment in rhinitic patients with pollen allergy was able to reduce symptoms, inflammatory cells, and costs better than symptomatic use.[13] Therefore continuous treatment leads to a significant reduction of inflammatory infiltration and results in better control of symptoms.

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